*Medical Progress*

# The Penicillins, Old and New

## Review and Perspectives

PAUL D. HOEPRICH, M.D., *Davis*

IT IS CONVENIENT, in reviewing developments that span many years, to break the time that has passed into decadal periods. Taking Fleming's discovery of penicillin in 1928 as the point for departure,[1] 1968 becomes the year of commencement of the fifth decade of penicillin (Table 1).

### Decade I

Following his original observation that staphylococci were inhibited from growth on a culture plate in the vicinity of a contaminating mold—subsequently identified as *Penicillium notatum*—Dr. Fleming prepared extracts of broth cultures of his strain of *P. notatum*. He named his crude material penicillin and observed its remarkable antibacterial activity and virtual lack of toxicity to experimental animals. Although Fleming continued to work with penicillin and write about his discovery, he attracted little interest. About the only use made of penicillin during this first decade was as an additive to culture media to facilitate isolation of *Hemophilus* sp.

### Decade II

In 1938, a group of investigators, headed by Dr. Howard Florey, undertook study of penicillin at the Sir William Dunn School of Pathology at Oxford. The properties of extraordinary antibacterial potency with seeming absence of toxicity for animals, as noted by Fleming, were confirmed and extended.[2] Despite the exigencies of World War II, the work continued and the Oxford group developed production of penicillin to a practical level that allowed clinical trial. With proof of therapeutic efficacy, there was obvious need for mass production of penicillin—a development impossible in embattled Britain. In July, 1941, the focus of research on penicillin shifted to the Northern Regional Research Laboratory of the U.S. Department of Agriculture in Peoria, Illinois.

With alteration of the culture medium, selection of naturally occurring variants, application of mutagens with further selection, the yield of penicillin from *P. notatum* was brought to 150-200 units per ml from a starting level of 2 to 5 units per ml. No further improvement in yield from *P. notatum* seemed possible; moreover, since surface growth of the mold was necessary, real efficiency in production was not possible. Deep tank, submerged culture had to be employed for reduction in production costs but *P. notatum* made very little penicillin when grown submerged in liquid media.

Screening of isolates of *Penicillium* sp. from soil samples collected from all over the world failed to turn up a variant that would grow submerged and make penicillin. However, in 1944 a strain of *Penicillium chrysogenum* isolated from a moldy cantaloupe found in a produce market in Peoria was found to produce around 260 units per ml growing in submerged culture. Irradiation and exposure to radiomimetic chemicals, with selection, led to the presently used descendants of the Peoria cantaloupe strain of *P. chrysogenum* that yield over 3,000 units per ml of culture medium.

There was a notable perturbation in 1945 soon after a high-yield mutant was put into production of penicillin. The penicillin that was produced was measured in units of potency by conventional *in*

From the Section of Clinical Microbiology and Immunology, Department of Internal Medicine, School of Medicine, University of California, Davis and the Sacramento County Hospital, Sacramento.

Submitted 23 August 1968.

Reprint requests to: Section of Clinical Microbiology and Immunology, Department of Internal Medicine, School of Medicine, University of California, Davis 95616.

TABLE 1.—*The year 1968 is the beginning of the fifth decade since Fleming's discovery of penicillin*

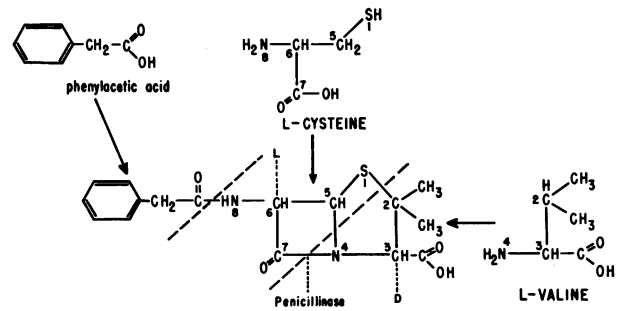
| *Decade* | *Event* | *Application* |
|---|---|---|
| I 1928-1938 | Discovery | Additive to culture media |
| II 1938-1948 | Development | Production<br>Purification<br>Therapeutic application |
| III 1948-1958 | Directed biosynthesis | Recognition and preparation of discrete penicillins: |
| IV 1958-1968 | Chemical synthesis and semi-synthesis | acid resistant<br>penicillinase resistant<br>altered spectrum |
| V 1968- | ? | Design and preparation of penicillins with a limited range of antimicrobial activity |

*vitro* assay. But when it was used to treat infections the mutant-produced penicillin was less effective, unit for unit, than the parent-mold penicillin. It was soon found that the difficulty related to the fact that there was not a penicillin, but, in fact, penicillins. The new mutant made principally penicillin K (n-heptylpenicillin), a penicillin even more active in the test tube than penicillin G (benzylpenicillin). However, when given in therapy, penicillin K apparently entered into such firm binding with proteins of the host that it was unavailable for antibacterial action. The situation improved dramatically when it was discovered that high yield could be maintained, but deviated to the production of penicillin G by supplying phenylacetic acid in the culture medium. Such directed biosynthesis of penicillin G was possible because the final step in the biosynthesis of any penicillin is acylation—the addition of an organic acid to the primary amino group of the nucleus that is characteristic of all penicillins (Chart 1).

## Decade III

It was a short step to trial of other organic acids, not known to be components of natural penicillins. In this way, biosynthesis was directed to yield penicillin V (phenoxymethylpenicillin), by addition of phenoxyacetic acid to the culture medium. The first penicillin that was resistant to degradation by acid was the result and reliable peroral therapy with a penicillin was the consequence.

Another example of directed biosynthesis was penicillin O (allylthiomethylpenicillin). Heralded as a kind of penicillin which would be safe for use in patients with demonstrated hypersensitivity to penicillin G, penicillin O is of historical interest.




BENZYLPENICILLIN
*m.w. 333.37*

**Chart 1.—The biosynthesis of 6-aminopenicillanis acid, the bicyclic dipeptide nucleus common to all penicillins, results from condensation of L-cysteine with L-valine. Note that inversion of the optical activity of carbon atom 3 (valine) results on condensation. Acylation with phenylacetic acid completes the synthesis of benzylpenicillin.**

Since penicillin O also engendered hypersensitivity reactions, it became clear that alteration in the side-chain that differentiates one penicillin from another does not alter the allergenic nature of the molecule.

The biosynthetic origins of the basic penicillin nucleus were deciphered in 1957.[3] As is shown in Chart 1, 6-aminopenicillanic acid, the bicyclic dipeptide that is the penicillin nucleus, is made from the amino acids L-cysteine and L-valine. Curiously, the optically active carbon atom of the L-valine precursor is inverted during condensation —a fact of enormous importance to chemical synthesis of penicillins.

## Decade IV

In 1959, Sheehan and Henery-Logan reported the total synthesis of penicillin V.[4] In the course of laboratory synthesis, all possible stereoisomers of penicillin V were produced. It was proved beyond doubt that the centers of optical activity in the 6-aminopenicillanic acid nucleus had to be precisely the way the *Penicillium* mold oriented these carbon atoms during the biosynthesis of penicillins in nature. It became apparent then, as a result of the total synthesis of penicillin, that the only part of the penicillin molecule which was susceptible to alteration was the side chain. Two other events occurred at about this same time that also had bearing on semi-synthesis.

Batchelor, in Great Britain, isolated a variant of *Penicillium chrysogenum,* which, in a special culture medium, synthesized 6-aminopenicillanic acid without any side chain attached to it.[5] One year

TABLE 2.—*Comparison of Penicillins*

| Penicillin | Resistance to | | Potency | | | | Versus | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Staphylococcus aureus | | | | | | | |
| | Penicillinase | Acid | Pcn'ase + | | Pcn'ase − | | Strep. pyogenes Group A | | S. typhi | |
| Benzyl (G) | Low 10 | Low 5 | Low | 0 | High | 100 | High | 100 | Low | 25 |
| Phenoxymethyl (V) | Low 10 | Mod. 60 | Low | 0 | High | 100 | High | 100 | Low | 10 |
| Methicillin* | High 100 | Low 0.3 | Mod. | 25 | Low | 5 | Low | 5 | Low | 0 |
| Oxacillin† | High 80 | Mod. 60 | High | 100 | Mod. | 50 | Low | 15 | Low | 5 |
| Nafcillin† | High 80 | Mod. 60 | High | 100 | Mod. | 50 | High | 80 | Low | 5 |
| Cloxacillin† | High 80 | Mod. 60 | High | 100 | Mod. | 50 | High | 80 | Low | 5 |
| Dicloxacillin† | High 80 | Mod. 60 | High | 100 | Mod. | 50 | High | 80 | Low | 5 |
| Ampicillin | Low 10 | High 100 | Low | 2 | Low | 15 | Low | 15 | High | 75 |

*Methicillin has no place in modern therapeutics.
†While similar in many respects, these agents do differ significantly in:
(1) Efficiency of absorption from the gastrointestinal tract: dicloxacillin > [oxacillin=cloxacillin] > nafcillin.
(2) Potency against non-staphylococcal Gram + cocci: [nafcillin=cloxacillin=dicloxacillin] > oxacillin.
(3) That is: for parenteral injection nafcillin and cloxacillin are interchangeable (do not use oxacillin); for oral administration, dicloxacillin is preferable (do not use nafcillin).

later, Sakaguchi and Murao reported the liberation of 6-aminopenicillanic acid from penicillin G by an amidase isolated from another strain of *P. chrysogenum*.[6] The work of Sakaguchi and Murao was confirmed in other laboratories, and other fungi were found capable of elaborating acylases active against various penicillins.[7] As a result, enormous supplies of 6-aminopenicillanic acid derived from *Penicillium* fermentation became available and the preparation of penicillins by the chemical addition of side chains—that is, semi-synthesis—was commercially feasible.

There were three major goals to semi-synthesis. Not mutually exclusive and not yet completely realized, these were the preparation of penicillins that were:

(1) Resistant to inactivation by β-lactamases, for example the penicillinase of *Staphylococcus aureus;*

(2) Resistant to degradation by acid;

(3) Significantly altered in antibacterial spectrum from penicillin G. The present state of semi-synthetic penicillins will be considered according to these goals.

### *β-lactamase resistance*

Methicillin, the first semi-synthetic, penicillinase-resistant penicillin, remains the most resistant penicillin to inactivation by β-lactamases thus far prepared. However, other deficiencies are so great as to relegate methicillin to the dustbin of history (see Table 2). Not only are oxacillin, nafcillin, cloxacillin and dicloxacillin resistant to β-lactamases, but also, these semi-synthetic penicillins resist degradation by acid and are measurably more active antimicrobics. Dicloxacillin is the most efficiently absorbed of the penicillinase-resistant penicillins and so is preferable for peroral treatment of infections caused by penicillinase-producing *Staphylococcus aureus* that are not life-threatening. Life-threatening infections caused by penicillinase-producing *S. aureus* are indication for parenteral therapy. Nafcillin and cloxacillin are interchangeable and are preferable, for both are measurably more active than oxacillin and methicillin, the only other semi-synthetic, penicillinase-resistant penicillins at present available for parenteral administration. Ampicillin is of no value in the treatment of staphylococcal infections. The penicillinase-resistant penicillins have but one indication—infection caused by penicillinase-producing *S. aureus.*

### *Acid resistance*

The variation in acid-lability among penicillins is great, ranging from the extreme in lability with methicillin to the extreme in stability with ampicillin (Table 2). While relative stability to acid will insure that active drug will pass into the duodenum after peroral administration, efficient absorption is not thereby guaranteed. For example, although ampicillin is the most resistant of available penicillins to degradation by acid, only 15 to 20 percent of a peroral dose attains to systemic distribution—see Chart 2. Although ampicillin is susceptible to inactivation by β-lactamases elaborated by enteric bacteria, it is probable that the apparently poor absorption that occurs is consequent on enterohepatic cycling. There is efficient hepatic removal of ampicillin from portal blood with excretion in the bile followed by reabsorption from the gut.[11] This is a phenomenon of clinical significance. For illustration:

A 52-year-old white man was admit-

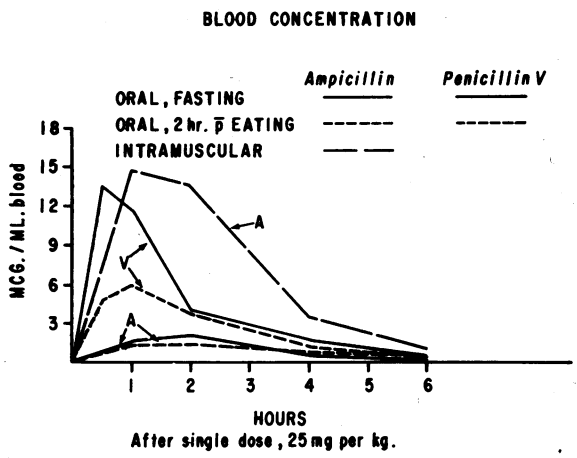



Chart 2.—As judged by penicillinemia, only 15 to 20 percent of a peroral dose of ampicillin attains to blood-borne systemic distribution.[8] Data regarding the same dosage of penicillin V are shown for comparison.[9,10]

ted for resection of a carcinoma of the greater curvature of the stomach that had been diagnosed at another hospital. At operation an 80 percent gastrectomy, omentectomy and splenectomy were performed to remove a large, ulcerated gastric carcinoma. The patient became febrile almost immediately after operation. Four days later a left subdiaphragmatic abscess was diagnosed and drained at operation the following day. After drainage, the patient was treated with cephalothin, 6 gm, intravenously, daily. Fever, averaging 102.5°F, persisted. On the fourth day after drainage, because the *Escherichia coli* isolated from the pus was susceptible to ampicillin, cephalothin was stopped and ampicillin, 0.5 gm by mouth every six hours, was started. Fever, toxicity, and copious drainage of pus persisted during the next four days. Oral therapy was then replaced by intramuscular injection of 2.0 gm of ampicillin every six hours. The temperature fell to normal in 24 hours; gradual return of well-being and decrement in drainage followed over several days. Oral candidiasis, symptomatic since the seventh postoperative day, responded to gargling with mystatin mouth wash.

*Altered spectrum*

Among Gram-positive cocci, the enterococcal group of streptococci are notably resistant to benzylpenicillin. The standard treatment, a combination of benzylpenicillin and streptomycin, is often effective. However, there are disadvantages to the administration of two drugs, and the combination is not uniformly effective. By *in vitro* testing, the bactericidal potency of ampicillin against enterococci is significantly greater than that of benzylpenicillin.[12,13] There may be clinical reflection of these facts:

A 63-year-old white man was referred because of bacterial endocarditis caused by *Streptococcus fecalis,* consequent on transurethral resection of the prostate five months earlier. Because of a history of allergic sensitivity to penicillin, initial therapy at another hospital included various non-penicillin antibiotics, and cephalothin, in large doses. There was defervescence, but persistence of malaise, increasing confusion and disorientation. In addition, blood cultures obtained while the patient was receiving cephalothin and chloramphenicol, the regimen of antimicrobics that was being given at the time of referral, yielded *S. fecalis.* There was also failure of bactericidal action against the *S. fecalis* isolated from the patient by undiluted serum obtained from the patient during treatment.

On admission, the confused, quasi-oriented, rather wasted patient had, also, a holosystolic apical murmur radiating to the axilla, mild anemia, and an elevated sedimentation rate. Blood cultures yielded *S. fecalis.*

Ampicillin was bactericidal by *in vitro* test, at 0.01 μGm per ml, while penicillin G and cephalothin had no bactericidal activity. Intradermal and intramuscular tests with ampicillin yielded no reaction. Accordingly, treatment was started with ampicillin, 10 gm daily by continuous intravenous infusion. At this dose, however, the patient's serum did not show bactericidal activity against *S. fecalis.* Ampicillin was increased to 20 gm daily; in addition, probenecid 2.0 gm a day by mouth, and vancomycin 2.0 gm a day, intravenously, were given. On this regimen, the bactericidal activity demonstrable at a 1:2 dilution of the patient's serum could be abolished by addition of penicillinase.

He was treated for six weeks and was well and active when last observed, a year and a half after treatment.

While *Listeria monocytogenes* are susceptible to ampicillin by *in vitro* testing, benzylpenicillin is significantly more active.[14] Trial of ampicillin in the treatment of listeriosis has been too meager to permit evaluation.

Significant change in antibacterial spectrum, as compared with benzylpenicillin, has been claimed for ampicillin with regard to the Gram-negative bacilli *Hemophilus influenzae, Escherichia coli,*

TABLE 3.—*Comparison of the susceptibility of isolates of* Hemophilus influenzae *from the cerebrospinal fluid*[17,18]

| *Authors, date* | *μGm. per ml. of* | |
|---|---|---|
| | *Benzylpenicillin* | *Ampicillin* |
| Ivler *et al.*, 1963 | 0.10-1.6* 126 isolates | 0.10-1.6* 126 isolates |
| Barnett *et al.*, 1966 | 0.20-1.56† 15 isolates | 0.025-0.78† 41 isolates |

*Minimal bactericidal concentrations.
†Minimal inhibitory concentrations.

*Salmonella* sp., *Shigella* sp. and *Proteus mirabilis*. These claims will be examined in the order listed.

*Hemophilus influenzae*. By *in vitro* testing of the susceptibility of isolates from the cerebrospinal fluid, there was no difference in the bactericidal potency of benzylpenicillin and ampicillin (see Table 3). The treatment of meningitis caused by *H. influenzae* has been evaluated in three controlled studies that permit comparison of ampicillin as the sole antimicrobic with chloramphenicol given alone or with a sulfonamide and/or benzylpenicillin.[16-18] It is clear that ampicillin alone, given by intravenous injection in a dose of 150 to 400 mg per kg of body weight per day, is quite as effective as multi-agent therapy (Table 4).

In view of the identity in bactericidal potency of benzylpenicillin and ampicillin by *in vitro* test, it is curious that success with benzylpenicillin comparable to that obtained with ampicillin has not been reported. Very likely the difficulty is no more than inadequacy of dosage with benzylpenicillin. For example, Zinnemann in 1946[19] probably gave as much as 2 to 20 mg of benzylpenicillin per kg of body weight per day by intramuscular injection, along with 6 to 38 mg injected into the lumbar subarachnoid space. Supporting the notion of inadequate dosage is the report of Howe[20] describing inadvertent cure of meningitis caused by *H. influenzae* but treated with benzylpenicillin (12 to 135 mg per kg body weight per day by intramuscular injection) under the mistaken diagnosis of meningococcal meningitis.

*Escherichia coli*. About 80 percent of clinical isolates of *E. coli* are inhibited from growth *in vitro* by concentrations of ampicillin that are relevant to therapy[21-23]—a situation matched by benzylpenicillin.[23] Urinary tract infections caused by *E. coli* can usually be successfully treated with either kind of penicillin[22,24,25] because these infections are usually infra-nephric and penicillins are concentrated in the urine as a consequence of excretion.

Life-threatening infections with *E. coli* are best treated with another antimicrobic agent that has greater probability of bactericidal effectiveness unless there is certainty that the *E. coli* causing the infection is killed *in vitro* by 25 μGm (or less) ampicillin per ml. The following report is illustrative:

> A 77-year-old white woman was admitted with pneumonia. She had a history of repeated pulmonary infections and severe rheumatoid arthritis. Initial therapy with penicillin G, directed against the staphylococcus isolated from the sputum, was associated with clinical improvement. However, two weeks after admission, after penicillin had been discontinued for several days, an x-ray film of the chest showed a new infiltrate. Transtracheal aspiration of lower respiratory tract secretions yielded large numbers of *Hemophilus influenzae*. Ampicillin therapy, 1 gm, intramuscularly every eight hours, was begun. Despite clinical and radiologic evidence of improvement of the pulmonary infection, the patient remained mildly febrile. Blood and urine cultures were sterile. On the fifteenth day of ampicillin therapy, the temperature suddenly rose to 105°F; hypotension intervened and the patient died. Blood and sputum cultures taken premortem yielded *Escherichia coli*.

*Salmonella* sp. According to *in vitro* study, both typhoidal and non-typhoidal species of *Salmonella* are susceptible to ampicillin.[26-28] Indeed, in the test tube, ampicillin is more active than

TABLE 4.—*Ampicillin employed alone in the treatment of meningitis caused by* Hemophilus influenzae *was at least as effective as multi-agent regimens*[16-18]

| *Authors, date (dosage)* | *Mortality* | *Sequelae* |
|---|---|---|
| Mathies *et al.*, 1965 (150 mg./kg./day) | 4/66 = 6.1%* (10/107 = 9.3%)† | 6/66 = 9.0%* (11/107 = 10.2%)† |
| Barrett *et al.*, 1966 (150 mg./kg./day) | 1/16 = 3.5%* (2/12 = 16.7%)† | 4/16 = 25%* (5/12 = 41.7%)† |
| Fleming *et al.*, 1967 (400 mg./kg./day) | 1/21 = 4.8%* (1/20 = 5.0%)† | ? |

*Ampicillin therapy.
†Chloramphenicol, 100 mg per kg body weight per day by intravenous injection, with or without sulfonamide and/or benzylpenicillin, for one day.

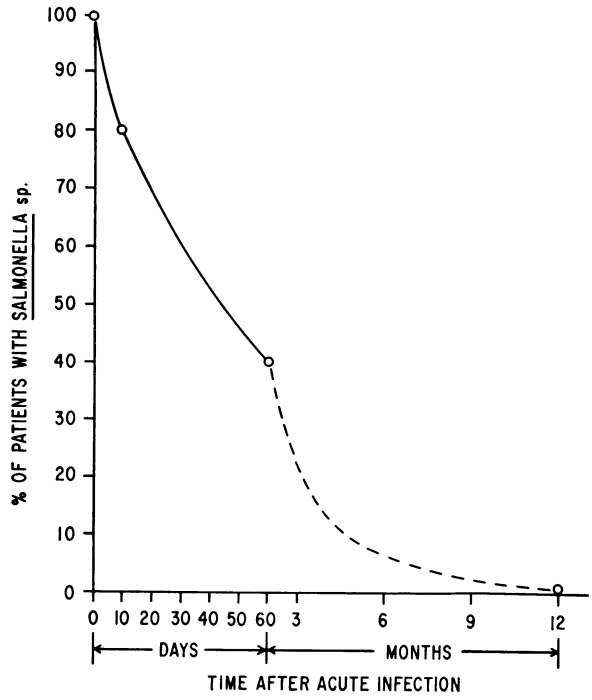



**Chart 3.—Following subsidence of acute clinical illness, there is spontaneous cessation of excretion of the infecting *Salmonella* sp. in an essentially exponential decline with time. True carriers, those who continue shedding one year after acute disease, range from 2 to 5 percent with *Salmonella typhi* to less than 1 percent with non-typhoidal *Salmonella* sp.**

either benzylpenicillin or chloramphenicol against *Salmonella typhi*. In addition, the pharmacologic property of excretion by the liver with actual concentration in the bile led to speculation that ampicillin might have particular merit in dealing with carriers of *Salmonella* sp.

There is now sufficient experience to indicate that peroral therapy with ampicillin is inferior to peroral therapy with chloramphenicol in the treatment of both acute typhoid fever and non-typhoidal salmonellosis.[8,28-37] In one clinical trial, ampicillin given by injection (ten patients) was as effective as chloramphenicol given by injection (ten patients) in treatment of typhoid fever.[38] However, in another study, none of five patients with non-typhoidal salmonellosis were cured by parenteral administration of ampicillin.[39]

Following subsidence of acute salmonellosis, there is natural, spontaneous cessation of excretion of bacilli according to a pattern approximating exponential decay (Chart 3). For this reason, valid evaluation of the effect of any regimen of treatment of carriers of *Salmonella* sp. must refer to true carriers—those who continue to shed *Salmonella* sp. one year after clinical recovery from acute salmonellosis. The incidence of true carriers following typhoid fever is 2 percent to 5 percent; following non-typhoidal salmonellosis, less than 1 percent. The presence of biliary tract disease, with or without cholelithiasis, is conducive to the establishment of a carrier state. However, persons who appear to have normal biliary tracts may become true carriers. Chloramphenicol has not been effective in the treatment of true carriers of *Salmonella* sp.

Since the only known host to *S. typhi* is the human, many investigators have assessed the utility of ampicillin to the treatment of the true typhoid carrier. Referring only to those reports dealing with three or more true carriers, treatment with ampicillin was apparently successful with 49 of 79 patients.[40-49] On the whole, the probability of success was enhanced if there was no biliary disease, or if a diseased gallbladder was removed. High dosage, 4 to 6 gm per day for four to six weeks, supplemented with probenecid, 2 gm per day, also made for success in terminating carriage of *S. typhi*.

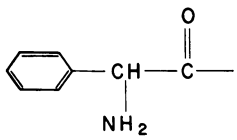
Carriers of non-typhoidal *Salmonella* sp. have been less well studied. However, reports of 14 carriers treated with ampicillin tell of but two cures.[37,39,40-42,46,48]

*Shigella* sp. With increasing frequency clinical isolates of *Shigella* sp. are resistant to the sulfonamides as well as to other antimicrobic agents. Therefore it is of clinical significance that the effectiveness of ampicillin in the treatment of bacillary dysentery has been documented.[8,50,51]

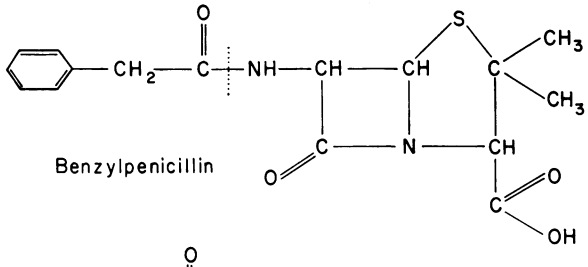
*Proteus mirabilis*. Of the *Proteus* sp., only *P. mirabilis* is susceptible to ampicillin. The degree of susceptibility to ampicillin is not remarkably greater than that displayed to benzylpenicillin.[52] Since high dosage parenteral therapy is required with either kind of penicillin (8 to 25 gm per day) for successful treatment of renal or extra-renal infections caused by *P. mirabilis*, cost alone dictates preference for benzylpenicillin.

## Decade V

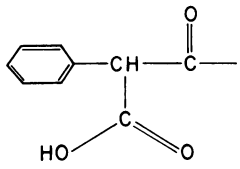
What of the future of penicillins? Surely the reagent shelves of the chemists are not yet bare insofar as side-chain variation is concerned. However, with the logging of more than 2,000 semisynthetic penicillins, differing only in side-chain structure,[53] it might seem that the probability of preparation of additional useful penicillins through



Ampicillin



Benzylpenicillin



Carbenicillin

**Chart 4.—Carbenicillin, the newest of the semi-synthetic penicillins to come to clinical investigation, like ampicillin, is a variant of benzylpenicillin. However, carbenicillin and ampicillin are antipodean variants for the carboxyl grouping of the former is electronegative whereas the primary amino grouping of the latter is electropositive.**

acylation is becoming remote. Yet, the recent reports of effectiveness against *Pseudomonas aeruginosa* of the semi-synthetic penicillin, carbenicillin[54,55] may indicate that there is more to come. In structure, carbenicillin is as radically deviant from ampicillin as it can be (see Chart 4), for the primary amino grouping of the ampicillin side chain is replaced with a carboxyl group. The trend in semi-synthesis that may be illustrated by carbenicillin is to penicillins with a narrow range of effectiveness—penicillins selected to deal with relatively specific, problem groups of bacteria. Carbenicillin is nicely illustrative for it promises to be useful against *Pseudomonas* sp., *Providencia* sp. and perhaps the indole positive *Proteus* sp.[55,56]

## Conclusion

There is no more dramatic story than that of the penicillins, for no other event has so profoundly and irrevocably changed clinical medicine. In tracing the path from discovery to the present, recent developments have been weighted, since this is the area of penicillin yet in the flux of investigation. The story is not yet ended and a direction that might be followed in the fifth decade of penicillin has been indicated.

## REFERENCES


1. Fleming, A.: On the antibacterial action of cultures of a penicillium with special reference to their use in the isolation of B. influenza, Brit. J. Exp. Path., 10:226-236, 1929.

2. Chain, E., Florey, H. W., Gardner, A. D., Heatley, N. G., Jennings, M. A., Orr-Ewing, J., and Sanders, A. G.: Penicillin as a chemotherapeutic agent, Lancet, 2:226-288, 1940.

3. Arnstein, H. R. V.: The biosynthesis of penicillin and some other antibiotics, Ann. Report Chem. Soc. (London), 54:339-352, 1957.

4. Sheehan, J. C., and Henery-Logan, K. R.: The total synthesis of penicillin V, J. Am. Chem. Soc., 79:1262-1263, 1957.

5. Batchelor, F. R., Doyle, F. P., Nayler, J. H. C., and Robinson, G. N.: Synthesis of penicillin:6-aminopenicillanic acid in penicillin fermentations, Nature, 183:257-258, 1959.

6. Sakaguchi, K., and Murao, S.: A new enzyme, penicillin-amidase —Preliminaries, J. Agr. Chem. Soc. (Japan), 23:411, 1950.

7. Hamilton-Miller, J. M. T.: Penicillinacylase, Bact. Rev., 30:761-771, 1966.

8. Ross, S., Lovrien, E. W., Zaremba, E. A., Bourgeois, L., and Paig, J. R.: Alpha-amino-benzylpenicillin—A new broad spectrum antibiotic—Preliminary clinical and laboratory observation, JAMA, 182:238-242, 1962.

9. Cronk, G. A., Wheatley, W. B., Fellers, G. F., and Albright, H.: The relationship of food intake to the absorption of potassium alphaphenoxyethyl penicillin and potassium phenoxymethyl penicillin from the gastrointestinal tract, Am. J. Med. Sci., 240:219-225, 1960.

10. Griffith, R. S.: Comparison of antibiotic activity in sera after the administration of three different penicillins, Antibiotic Med. and Clin. Ther., 7:129-135, 1960.

11. Stewart, G. T., and Harrison, P. M.: Excretion and re-excretion of a broad-spectrum penicillin in bile, Brit. J. Pharm., 17:414-419, 1961.

12. Simon, H. J.: Antimicrobial susceptibility of Group D hemolytic streptococci (enterococci), Am. J. Med. Sci., 253:14-18, 1967.

13. Sonne, M., and Jawetz, E.: Comparison of the action of ampicillin and benzylpenicillin on enterococci in vitro, Appl. Microbiol., 16:645-648, 1968.

14. Nelson, J. D., Shelton, S., and Parks, D.: Antibiotic susceptibility of *Listeria monocytogenes* and treatment of neonatal listeriosis with ampicillin, Acta Paed. Scand., 56:151-158, 1967.

15. Ivler, D., Thrupp, L. D., Leedom, J. M., Wehrle, P. F., and Portnoy, B.: Ampicillin in the treatment of acute bacterial meningitis, *In* Antimicrobial Agents and Chemotherapy—1963, Am. Soc. Microbiol., Ann Arbor, Mich., 1964, pp. 335-345.

16. Barrett, F. F., Eardley, W. A., Yow, M. D., and Leverett, H. A.: Ampicillin in the treatment of acute suppurative meningitis, J. Pediat., 69:343-353, 1966.

17. Mathies, A. W., Jr., Leedom, J. M., Thrupp, L. D., Ivler, D., Portnoy, B., and Wehrle, P. F.: Experience with ampicillin in bacterial meningitis, *In* Antimicrobial Agents and Chemotherapy—1965, Am. Soc. Microbiol., Ann Arbor, Mich., 1966, pp. 610-617.

18. Fleming, P. C., Murray, J. D. M., Fujiwara, M. W., Prichard, J. S., and McNaughton, G. A.: Ampicillin in the treatment of bacterial meningitis, *In* Antimicrobial Agents and Chemotherapy—1966, Am. Soc. Microbiol., Ann Arbor, Mich., 1967, pp. 47-52.

19. Zinnemann, K.: Survey of the outcome of 20 cases of *H. influenzae* meningitis related to bacterial type, Brit. Med. J., 2:931-936, 1946.

20. Howe, C.: *Hemophilus influenzae* meningitis—Report of two cases in adults, Ann. Int. Med. J., 47:141-145, 1957.

21. Rolinson, G. N., and Stevens, S.: Microbiological studies on a new broad spectrum penicillin, "Penbritin," Brit. Med. J., 2:191-196, 1961.

22. Anderson, K. N., Kennedy, R. P., Plorde, J. J., Shulman, J. A., and Petersdorf, R. G.: Effectiveness of ampicillin against gram-negative bacteria, JAMA, 187:555-561, 1964.

23. Hoeprich, P. D., Watanabe, F., and Parker, R. H.: Grouping of Gram negative bacteria and susceptibility to antimicrobial agents, *In* Antimicrobial Agents and Chemotherapy—1965, Am. Soc. Microbiol., Ann Arbor, Mich., 1966, pp. 412-416.

24. Brumfitt, W., Percival, A., and Carter, M. J.: Treatment of urinary tract infections with ampicillin—A clinical trial, Lancet, 1:130-133, 1962.

25. Stamey, T. A., Govan, D. E., and Palmer, J. M.: The localization and treatment of urinary tract infections—The role of bactericidal urine levels as opposed to serum levels, Medicine, 44:1-36, 1965.

26. Kaye, D., Merselis, J. G., and Hook, E. W.: Susceptibility of *Salmonella* species to four antibiotics, New Eng. J. Med., 269:1084-1086, 1963.

27. Parker, R. H., Watanabe, F., and Hoeprich, P. D.: Susceptibility of *Salmonella heidelberg* isolated during the Utah epidemic of 1964, *In* Antimicrobial Agents and Chemotherapy—1964, Am. Soc. Microbiol., Ann Arbor, Mich., 1965, pp. 423-432.

28. Robertson, R. P., Wahab, M. F. A., and Raasch, F. O.: Evaluation of chloramphenicol and ampicillin in salmonella enteric fever, New Eng. J. Med., 278:171-176, 1968.

29. Kennedy, W. P. U., Wallace, A. T., and Murdoch, J. M.: Ampicillin treatment of certain gram-negative bacterial infections, Brit. Med. J., 2:962-965, 1963.

30. Patel, K. M.: Ampicillin in typhoid fever, Brit. Med. J., 1:907, 1964.

31. Uwaydah, M., and Shamma'a, M.: The treatment of typhoid fever with ampicillin, Lancet, 1:1242-1243, 1964.

32. Scioli, C., Giusti, G., and Balestrieri, G.: Comparison of ampicillin and chloramphenicol in treatment of typhoid fever, Postgrad. Med., Suppl., 40:87-91, 1964.

33. Manriquez, L., Salcedo, M., Borgono, J. M., Marzullo, E., Kraljevic, R., Parades, L., and Valdivieso, R.: Clinical trials with ampicillin in typhoid fever and paratyphoid A, Brit. Med. J., 2:152-153, 1965.

34. Sanders, W. L.: Treatment of typhoid fever—a comparative trial of ampicillin and chloramphenicol, Brit. Med. J., 2:1226-1227, 1965.

35. Dawkins, A. T., Jr., and Hornick, R. B.: Evaluation of antibiotics in a typhoid model, *In* Antimicrobial Agents and Chemotherapy—1966, Am. Soc. Microbiol., Ann Arbor, Mich., 1967, pp. 6-10.

36. Sleet, R. A., and Sangster, G.: Comparison of ampicillin and chloramphenicol in treatment of paratyphoid fever, Brit. Med. J., 1:148-150, 1964.

37. Pettersson, T., Klemola, E., and Wager, O.: Treatment of acute cases of Salmonella infection and Salmonella carriers with ampicillin and neomycin, Acta Med. Scand., 175:187-190, 1964.

38. Kaye, D., Rocha, H., Eyckmans, L., Pratal, A., and Hook, E. W.: Comparison of parenteral ampicillin and parenteral chloramphenicol in the treatment of typhoid fever, Ann. N. Y. Acad. Sci., 145:423-428, 1967.

39. Parker, R. H., and Hoeprich, P. D.: Parenteral sodium ampicillin therapy of endocarditis, salmonellosis, and other bacterial infections, *In* Antimicrobial Agents and Chemotherapy—1965, Am. Soc. Microbiol., Ann Arbor, Mich., 1966, pp. 618-626.

40. Tynes, B. S., and Utz, J. P.: Factors influencing cure of salmonella carriers, Ann. Int. Med., 57:871-882, 1962.

41. Bullock, W.: Ampicillin therapy of salmonella carriers — a summary of laboratory and clinical observations, Am. J. Med. Sci., 246:42-47, 1963.

42. Münnich, D., Uri, J., and Valu, G.: Das kombinierte langfristig-hochdosierte Ampicillin- und Probenecid-Behandlung der Typhus-Bakterien-Dauerausscheider, Chemother., 8:226-240, 1964.

43. Christie, A. B.: Treatment of typhoid carriers with ampicillin, Brit. Med. J., 1:1609-1611, 1964.

44. Whitby, J. M. F.: Ampicillin in treatment of *Salmonella typhi* carriers, Lancet, 2:71-72, 1964.

45. Troy, P.: Ampicillin for typhoid carriers, Brit. Med. J., 1:1252-1253, 1964.

46. McFadzean, A. J. S., and Ong, G. B.: Intrahepatic typhoid carriers, Brit. Med. J., 1:1567-1571, 1966.

47. Simon, H., and Miller, A. C.: Ampicillin in the treatment of chronic typhoid carriers, New Eng. J. Med., 274:807-815, 1966.

48. Perkins, J. C., Devetski, R. L., and Dowling, H. F.: Ampicillin in the treatment of *Salmonella* carriers, Arch. Int. Med., 118: 528-533, 1966.

49. Kaye, D., Merselis, J. G., Jr., Connolly, C. S., and Hook, E. W.: Treatment of chronic enteric carriers of *Salmonella typhosa* with ampicillin, Ann. N. Y. Acad. Sci., 145:429-435, 1967.

50. Nelson, J. D., and Haltalin, K. C.: Broad-spectrum penicillins in enteric infections of children, Ann. N. Y. Acad. Sci., 145:414-422, 1967.

51. Haltalin, K. C., Nelson, J. D., Hinton, L. V., and Kusmiesz, H. T.: Double-blind treatment study of shigellosis comparing neomycin and ampicillin, *In* Antimicrobial Agents and Chemotherapy—1967, Am. Soc. Microbiol., Ann Arbor, Mich., 1968, in press.

52. Barber, M., and Waterworth, P. M.: Antibiotic sensitivity of *Proteus* species, J. Clin. Path., 17:69-74, 1964.

53. Sheehan, J. C.: The chemistry of synthetic and semi-synthetic penicillins, Ann. N. Y. Acad. Sci., 145:216-221, 1967.

54. Acred, P., Brown, D. M., Knudsen, E. T., Rolinson, G. N., and Sutherland, R.: New semi-synthetic penicillin active against Pseudomonas pyocyanea, Nature, 215:25-30, 1967.

55. Brumfitt, W., Percival, A., and Leigh, D. A.: Clinical and laboratory studies with carbenicillin—A new penicillin active against *Pseudomonas pyocyanea,* Lancet, 1:1289-1293, 1967.

56. Janis, B., Evans, R. G., and Hoeprich, P. D.: Providence bacillus bacteremia and septicopyemia, Am. J. Med., in press.

## A DOUBLE SHIBBOLETH FOR CHRONIC ACTIVE HEPATITIS

"[In diagnosing chronic active hepatitis] the laboratory tests . . . , I think, are rather . . . distinctive. And the thing that's important about the laboratory tests is that simultaneously we have evidence of chronic liver disease, largely exemplified by the serum protein changes, and at the same time, evidences of acute hepatic inflammation, largely provided by the transaminase tests. So look for evidences of chronic liver disease and acute liver disease together. In our group, unless we find both of these features in a patient, we rarely make the diagnosis of chronic active hepatitis."

—TELFER B. REYNOLDS, M.D., Los Angeles
*Audio-Digest* Internal Medicine, Vol. 15, No. 17